

TriReagent RNA Isolation Protocol

*The key to good RNA prep is *minimization of freeze-thawing of tissue and RNA during prep and preservation.*

Homogenization (perform in the hood with goggles)

1. Homogenize tissue in 1mL TRI Reagent/50-100mg-tissue* in a blue eppi with a fresh pestle (sample volume not to exceed 10% volume of TRI Reagent). First homogenize at high speed in .5mL and then add the other .5mL and homogenize at lower speed to avoid splattering.

*Add a small volume of TRI Reagent first and homogenize with RNase-free drill-bit, then add the remainder

NOTE: For small amounts of tissue (25-40 polyps) do a 250uL or 500uL prep.

2. Incubate **OVERNIGHT** at 4C

Phase Separation

3. With a new pestle, crush up any remaining tissue and incubate at RT for 5 minutes.

4. Add 100uL (for original homogenization in 1mL TRI Reagent) BCP (bromochloropropane)/1mL TRI Reagent
Cover samples and shake vigorously 15 seconds

5. Incubate mixture at RT for 2-15 minutes

6. Centrifuge at 12,000g for 15 minutes at 4°C

RNA will be in upper aqueous phase

***Do a back-BCP wash if you feel it is particularly dirty

Precipitation

7. Transfer aqueous upper phase to a clean tube

*optional: save aqueous and interphases at 4°C for isolation of DNA or protein

8. Add 500uL (for original homogenization in 1 mL TRI Reagent) isopropanol to the aqueous phase and mix

9. Incubate at RT for 5-10 minutes (or overnight at -20C <--- optimal)

10. Centrifuge at 12,000g for 8 minutes at 4°C (RNA will form a gel-like or white pellet)

RNA Wash

10. Remove supernatant and wash RNA pellet by vortexing in 1mL 75% EtOH

Centrifuge for 30 seconds at 7,500g at 4°C

11. Remove EtOH and air dry pellet for 3-5 minutes (don't overdry).

repeat wash if necessary

12. Solubilize in RNase secure water. Move immediately to DNase step or store overnight at -80.

DNase Treatment

1. TURBO DNASE TREATMENT

2. Add 0.1 volume 10X TURBO DNase Buffer and 1 µL TURBO DNase to the RNA, and mix gently. (only for max 200ug total. If more, see TURBODNase kit instructions)

3. Incubate at 37°C for 20-30 min.

4. Add resuspended DNase Inactivation Reagent (typically 0.1 volume) and mix well.

5. Incubate 5 min at room temperature, mixing occasionally.

6. Centrifuge at 10,000 x g for 1.5 min and transfer the RNA to a fresh tube.

Sodium Acetate Back Precipitation

1. add 1/10th volume of 3 M sodium acetate to sample

2. add 2.5x volume 95% EtOH and mix well

3. incubate at -80 or on dry ice for 2hours, or at -80 overnight

4. spin down, wash with 70% EtOH

5. resuspend in dH2O

Storage

Aliquot and store samples at -80